ORIGIN AND BEHAVIOR OF THE NUCLEOLUS IN PLANTS

HAIG DERMEN

With plates 64 to 67

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A. INTRODUCTION

The Problem of the nucleolus is one which has attracted investigators for many years, but despite the large amount of work done on this nuclear body, the problem is far from solved. The origin, the development, the relation between nucleolus and chromosomes, and the nucleolar function are phases of the problem which have received attention, but there is little concerted opinion regarding them. That the nucleolus is an essential component of cells is evidenced by the fact that it is present, very probably, in all types of cells of all organisms, a fact which may be responsible for Wilson's question (1925) "whether the nucleolus may not play a more active and important part in cell metabolism than most writers have hitherto assumed."

The importance of a cell body is not necessarily dependent upon its continuity and permanence in a particular structural form, such as is characteristic of the chromosomes. Unlike the chromosomes the nucleolus possesses no constant structural form and is not even continuous, in the strict sense of the word, appearing and disappearing during both mitosis and meiosis. This peculiarity alone has perhaps caused a confusion in evaluating its significance, but when its universality is considered, it would appear that its function is one of vital import to the cell, and its appearance and disappearance in the cell cycle may be correlated with that function, which is probably of a metabolic nature.

The present work is primarily intended to determine the origin and to trace the development of the nucleolus through all the stages of mitotic and meiotic development of the nucleus in plants of diverse types and to see what relationship exists between the chromosomes and nucleolus.

Many of the recent investigations are concerned with the origin of the nucleolus and its relation to the chromosomes. Its function has been, and still is, a matter of speculation. Van Camp (1924) believes that nucleoli originate from the chromosomes at telophase in the form of small globules which later fuse into larger forms, but still later, during the development of the chromosomes, through a direct contact established between a continuous spireme and the nucleolus, the nucleolar substance flows back again over the chromosomes to be again dissociated during telophase. Zirkle (1928 and 1930), by developing specific fixatives, confirms the idea of direct transportation of nucleolar substance into the spireme and demonstrates the presence of this substance in a skeleton form in the anaphase chromosomes. Heitz (1931a and 1931b) offers evidence to show that the nucleolus originates from the satellite chromosomes and only from them, and formulates a theory that accounts for the symmetry of the nucleoli in number, size, position, and form in the telophase of the sister* (daughter) nuclei. Abele (1930) seems to believe that the nucleolar substance divides equally, as do the chromosomes, thus giving rise to nucleoli in sister nuclei that show symmetry in all respects and that "jeder Nukleolus hat seinen Partner in der anderen Tochterzelle."

As for the function of the nucleolus, Montgomery (1898) assumes that the nucleolar substance is connected with the nutritive processes of the cell and, as an assimilation organ, takes food from the cytoplasm. Meyer (Guillermond et al., 1933) believes that the nucleolus is composed of "ergastique" elements of the cell. It is commonly believed that the nucleolus is nothing but reserve food for the chromosomes which may build themselves from this supply. De Mol (1926), Zirkle (1928), and Fikry (1930) suggest that this body may break up into small particles and act as the carriers of hereditary stimuli from the genes through the cytoplasm. Further discussion of these theories, as well as others, will be found in the text.

In addition to a study of the origin and behavior of the nucleolus an attempt has been made in the present study to clarify some of the points concerning the structural behavior of the chromosomes as a group throughout their developmental cycle and to throw light on the question of the supposedly continuous spireme and on the physical relationship often observed between chromosomes and nucleolus. Some of the conclusions are based on observations made from both living and fixed material.

B. MATERIAL AND METHODS

To make a study of this nature as comprehensive as possible, it was thought essential that the observations be made on various plants that are unlike taxonomically. The following plants were used: (a) *Callisia* and *Yucca* (Monocotyledons); (b) *Paeonia* and *Hepatica* (Dicotyledons); (c) *Pinus* (Gymnosperm); (d) *Polystichum* (Pteridophyte); and (e) a species of *Spirogyra* (Thallophyte). Some observations were made from various other plants to see if there could be found any peculiarities not present in those mentioned above. The most intensive observations were made on *Callisia* and *Paeonia*.

It was thought advisable to supplement the study of fixed material with that of living material as an aid in interpreting the phenomena observed. Living material was subjected to various toxic fluids, and their effects on the protoplasm in general, and nucleolus in particular, were observed, and thus an interpretation of fixed objects could be arrived at.

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*The use of the terms "sister cells" and "sister nuclei" is suggested when cells or nuclei of the same "generation" are discussed, "daughter cells" and "daughter nuclei" being reserved for cases where derivation is implied. These terms are so used in this paper.

A fixative of 5% formalin and .5% chromic acid, in equal parts, was the principal killing and fixing solution used. This fixative seems to have been originally developed by Lewitzky (1931) and was recommended by Marshak (1931). The present author has made use of it with success. Its usefulness was proved by applying it to the delicate stigma hairs of *Callisia,* and it was found to cause less disturbance to the cell and its contents than any other reagents commonly used for cytological preparations. With this fixative the nucleolus takes a dark stain both with crystal violet-iodine and iron-alum haematoxylin. In the material fixed with this reagent and stained with crystal violet-iodine both chromosomes and nucleolus retain the stain remarkably well even after long washing in absolute alcohol. For the technical procedure with this fixative the reader is referred to my paper (Dermen, 1932). In addition to this method of preparation the Ehrlich-Biondi differential stain recommended by Van Camp (1924) was used. This is made up of Orange G, acid fuchsin, and methyl green. (For full details the reader is referred to Lee's Vade Mecum, p. 177, 1928). Blotting paper tests of this stain described in this book were found essential and, accordingly, the proportion of reagents could be varied to give the proper reaction.

C. MORPHOLOGY OF CHROMOSOMES IN RELATION TO THE NUCLEOLUS

In order to make clear the nature of the relationship which may exist between chromosomes and nucleolus, it was considered essential that first a thorough analysis of the chromosome morphology should be made. For this reason this phase of the problem was undertaken.

I. STRUCTURAL CONTINUITY OF CHROMOSOMES IN CYCLE. When the Lewitzky fixative is used, at no period of development are the chromosomes found aggregating into an unrecognizable mass. They always retain their linearity, and at prophase are found within the nuclear membrane as units grouped in the same relative positions which they occupied during anaphase. It seems that the individual chromosomes retain their particular forms (Figs. 48, 49, and 2) as they move to their respective poles during division, bent at their polar constriction points. The prophase chromosomes (Fig. 2) differ from those at anaphase (Fig. 49) only in that the former are more opened up to fill the space inside the nuclear membrane, while the latter are forced together somewhat into a more compact form in moving to the poles. This compactness is extreme at telophase (Figs. 6, 10, 11, 16, and 17). From this point on, as soon as the new nuclear membrane is formed, there take place reverse morphological changes. A new nuclear membrane encloses the closely grouped telophase chromosomes; then follows the expansion of this membrane, due possibly to expansion of the tightly pressed chromosomes which start to develop into longer and finer threads, to the increase of nuclear sap, and to the development of nucleoli in number and size, resulting in an increase of nuclear volume as a whole. In comparing specifically Figs. 2 and 49 (in Fig. 49 only upper polar group should be considered), prophase and anaphase respectively, one can not fail to be impressed by the similarity of the orientation of the chromosomes. The difference is that in Fig. 2 the chromosomes are longer, spiral and zigzag in appearance, while in Fig. 49 they are short and straight, tending to crowd together as they are forced to the pole, and appear single in structure. It was found, however, after critical study, that even at anaphase the chromosomes possess a dual structure. As in prophase (Fig. 2) and early metaphase (Fig. 29—in this figure the region of primary constriction is illustrated as faithfully as possible), anaphase chromosomes are found split lengthwise and even show twists in the chromatid threads, as illustrated in Figs. 7 and 30, and these twists become more pronounced in telophase, as shown in Fig. 8.

The chromosome contours at anaphase, as illustrated in Figs. 49-51, seem significant, and the interpretation may be that these chromosomes are split and their chromatids twisted together, as shown in Figs. 7, 8, and 30. Similarly, premeiotic chromosomes of microsporocytes show similar contours, indicating that they are already split before microsporocyte chromosomes go through characteristic meiotic development. The split nature of the chromosomes at this stage has been confirmed by a number of reputable cytologists, such as Sharp (1929) and Kaufman (1931), working with plants, and McClung (1928) and Robertson (1931), working with animals. Darlington (1932), however, still maintains that duality of the chromosomes at this stage is due to optical illusion, although previously (1926) he reported seeing chromosomes split at anaphase. This anaphasic split, as maintained by Sharp and others, seems to take place during the late prophase and metaphase. Such a split was seen in the Callisia satellite chromosomes, as illustrated in Fig. 5. The chromosomes at this stage, besides being fully split into distinct halves in readiness for mitotic division, also seem to show in each chromatid a secondary split, part of which, at least, could be distinguished if observed carefully. At the upper end split there were also observed chromomere-like structures in pairs. It may be remarked here that only rarely can these features be made out clearly. Delicate fixation and staining are primary requisites; and even with the best technique it requires special attention and considerable care on the part of the investigator that this feature may not be interpreted as an optical illusion.

Here may be considered the arrangement of the chromosomes on the metaphase plate in preparation for mitotic division. The metaphase chromosomes (*Callisia,* Fig. 48) have a characteristic orientation which is considered normal; they are arranged in circular fashion, polar (primary) constrictions pointed toward the middle of the plate, and homologous chromosomes tending frequently to show a secondary pairing. If homologues are not always adjacent, at least there seems to be a tendency for them to orient themselves in such a fashion as to facilitate synapsis during meiosis. There may be a strong affinity between homologues at their polar constriction points that will play some part in bringing them together and result in their close pairing at meiosis. Structurally the chromosomes of *Callisia* vary considerably. The total number, as reported by Sax (1932), is 12, of which 4 have approximately median constriction (Figs. 48 and 49), and 8 have subterminal constriction. Two of the latter possess satellites. No secondary constrictions are observed.

It is not uncommon to find in the literature references to a granular stage of nucleus during the resting stage. A careful study of Figs. 18 and 33 perhaps will throw some light on the exact nature of the nucleus at this stage. Fig. 33 represents the telophase of somatic chromosomes from *Paeonia.* The two sister nuclei are viewed from the side and illustrated more or less diagrammatically. At this stage, as was pointed out above, the chromosomes are found already split and showing fine threads, each one representing a chromatid. The appearance of granulation often observed at these stages seems to be due both to curvatures and to points of strongly stained areas (Fig. 18). In mitosis these chromosomes will further elongate and open up to develop finally into a structural make-up similar to that illustrated in Fig. 2. If this stage were viewed from a different angle, it would no doubt present an entirely different aspect. Therefore, if it is true that at prophase the chromosomes hold positions comparable to those at anaphase, then any possibility of their joining end to end into a continuous spireme is inconceivable. This point is further confirmed by the work of Sax and Anderson (1933) and others who show that chromosomes may interlock during meiosis, a situation which would be impossible if the chromosomes were continuous.

The conception of the continuity of the chromosomes in a spireme has probably been derived from impressions of oblique and polar views of nuclei of stages illustrated in Fig. 1, and especially of early stages similar to those in Fig. 18. (Fig. 1 is of the same stage as Fig. 2, except that in the former the nucleolus is included.) Even in a stage as late as Fig. 1 one can easily be misled concerning the continuity of the chromosomes, but with care they can clearly be made out as separate units. When root-tip sections were crushed under a cover-glass to flatten the cells, it was apparent that in all stages chromosomes invariably show linearity and are never broken up into scattered granules. Martens (1928), having made a similar study from living and fixed material, finds the nucleus is not granular in make-up but primarily reticulate and filamentous, and that there is no continuous spireme at the beginning of prophase; that "Le réseau interphasique n'est donc que l'ensemble des chromosomes télophasiques—à peine plus évolués—et donc les mailles filamenteuses sont reliées par d'autres filaments d'union," and confirms "la persistance—ou mieux, la continuité morphologique et génétique—des structures chromosomiques d'une cinèse à l'autre, au cours de l'interphase et du repos."

II. Chromosome and Nucleolar Relationship. It can be seen from the preceding description of chromosomes that the idea of a continuous spireme is incompatible with the facts, that the chromosomes retain a characteristic arrangement inside the nuclear membrane, and that the individuality of the chromosomes is maintained; therefore a direct flow of nucleolar material, postulated by Van Camp and many others, from the end of a chromosome through the rest of the chromosomes, becomes an impossibility.

Figure 1 shows a large central nucleolus around which the chromosomes are distributed. In this nucleus all the chromosomes are found, with one exception, free from any physical connection with the nucleolus. At the upper side of the nucleolus there was noted the only probable connection at the bend of a chromosome with the nucleolus. I was not able to discover, at this stage, the presence of a pair of satellites characteristic of this species of *Callisia* and determine their relationship with the nucleolus, and only once, after a long search, I found two satellite-like bodies, one on each side of the nucleolus, at a considerable distance from the chromosome ends viewed from the side of the nucleus in a position similar to the one illustrated in Fig. 2; however, these bodies may have been extra-nucleolar bodies which are described below.

Van Camp (1924), using the Ehrlich-Biondi differential stain, found an intermediate coloration on the portion of the chromosomes attached to the nucleolus and therefore concluded that there was a direct flow of nucleolar material over the chromosomes. Zirkle (1931), by using a selective fixation method, confirms this conclusion and finds that the nucleolar material which has flowed "into" the chromosome threads was fixed at anaphase in a skeleton form. The present author has been unable to confirm the findings of these investigators. In all the cells he has examined there was no sign of intermediate staining between any portion of the chromosomes and nucleolus. The nucleolus at all stages was always bright red and the chromosomes pale blue when material was stained with the Ehrlich-Biondi solution. Fig. 3 is a diagrammatic drawing of a prophase stage from differentially stained material where no such intermediate coloration could be observed. On the other hand, if Zirkle's contention is correct, then the anaphase chromosomes were expected to show some red granular framework. Fig. 6 is such an anaphase stage; the chromosomes were of the same pale blue color as in the prophase in Fig. 3. When *Paeonia, Pinus, Polystichum,* and other species were subjected to similar treatment, the results were the same as those found in *Callisia;* the differentiation was always perfect.

It is quite generally assumed that the nucleolus decreases in volume during the development of the chromosomes, and that it supplies material to the "emerging" chromosomes. The facts on hand, however, seem to indicate that, at least in the plants studied, the situation is not such as described above, and that there is no diminution in size of the nucleolus from the resting stage, when it reaches its maximum volume, until the end of prophase before the nuclear membrane disappears. This fact can be demonstrated clearly by studying meiotic stages of species in which only one nucleolus is generally found, derived, no doubt, from fusion of a number of small nucleoli to form a large one. In *Callisia* there are usually two nucleoli present in the earliest meiotic stage, but as a rule there is only one at later stages. When there are two at early leptotene stage (Fig. 19), they are always smaller than the single nucleoli in adjacent nuclei (Fig. 20). Even at these early stages there are very few cells with double nucleoli, and these unquestionably later result in one nucleolus by fusion. Figs. 21-24 represent the zygotene stage, where the chromosomes are considerably thicker than in Fig. 20 and Fig. 25 the satellite chromosome pair at late diakinesis just before the nuclear membrane disappears, which, in this species, always holds the nucleolus at its satellite end, as reported by Sax (1932). In comparing the sizes of nucleoli in Figs. 20 and 25 and comparing both with the inner nucleolus of Fig. 18, which represents a cell from the earliest stage of meiotic development resulting from the division of the archespores, one is impressed by the constancy in size of the nucleoli all through these developmental stages. Similarly, Fig. 57

shows the diagrammatic representation of the volumes of the two nucleoli from *Hepatica, a* sketched from a very early leptotene, and *b* from the diplotene stage, showing the same constancy in size of the nucleolus as in *Callisia.*

The assumption of transference of nucleolar material to the chromosomes seems to be based on the fact that at later stages the chromosomes take up stain more readily than at earlier stages, a fact which has led many investigators to believe that there is a correlation between the stainability of chromosomes at later stages and entire disappearance of the nucleolus by the time of chromosome division. In *Yucca,* illustrated in Figs. 55 and 56, it is shown that there is hardly any sign of decrease in volume of the nucleolus which is left out in the cytoplasm during meiosis. In general, it appears that the nucleolus reaches a maximum size at a very early stage in the nucleus soon after a nuclear membrane encloses the chromosomes; the size of the nucleolus remains constant all through further development of the nucleus to the very end of prophase, very soon after the nuclear membrane disappears; then the nucleolus generally disappears, the rate depending upon the species of plant, as will be indicated below.

Belar (1928) seems to be justified in stating that there is not sufficient ground for assuming that the nucleolus has any role in the process of building up the chromosomes by increasing their mass. It was shown that when the differential stain was applied at all stages, the chromosomes took a pale blue color, while the nucleolus stained bright red. On the other hand, when crystal violet-iodine, or even iron-alum haematoxylin, is used, the chromosomes throughout do not show the same intensity of coloration as when Ehrlich-Biondi stain is used. Therefore this discrepancy of staining intensity of the chromosomes does not seem to be due to their being less chromatic at earlier stages, and more so at later stages, but rather to their "thinness" or "thickness," depending upon their stage of development. Moreover, the stainability and the degree of retaining of the stain of the fine chromosome threads may be altered with different fixatives and stains.

III. SATELLITES AND SECONDARY CONSTRICTIONS. Belar (1928) states that there is no basis for assuming that there is any connection (physiological) between satellites and nucleoli, while, on the other hand, Heitz (1931a and 1931b) has given great importance to these bodies. Heitz's theory is that nucleolar bodies originate on and around the achromatic threads behind the satellites and at secondary constriction areas, and that the number of nucleoli at late telophase is the same as these satellite and constricted chromosomes. De Mol (1927) was perhaps the first to notice a correlation in the number of satellites and nucleoli. In the present paper is reported a complete study of the satellite and constriction situation in *Callisia, Paeonia,* and *Pinus,* in order to have a basis for analyzing the relationship of the nucleolus to these features.

The somatic chromosomes of *Callisia, Paeonia,* and *Pinus* were subjected to a detailed study to determine the presence and number of satellites and secondary constrictions. Pinus chromosomes were found to be devoid of typical globular satellites, but instead there were present five pairs of chromosomes with secondary constrictions which varied as to their position and length of achromatic threads, though apparently constant for each chromosome so characterized. Fig. 54 illustrates *Pinus Strobus* chromosomes at anaphase (only part of the 12 pairs of chromosomes in each half is shown), showing some of these constricted areas on the chromosomes. *Callisia* (Fig. 48) has a pair of small satellites attached to relatively long threads which were hardly noticeable in this figure but are generally of good size, as shown in Fig. 5. Four plants of *Paeonia* were studied, two of which were of the species *P. suffruticosa* and two of different species,—namely, *P. Delavayi alba* and *P. Woodwardii.* Fig. 50 (*a* and *b*), drawn from two adjacent sections, represents an anaphase from *P. suffruticosa* which was cut into two by the microtome knife. The exact number of the satellites in this species was determined from this, as well as from other cells, and was found to be four, the position of which can be clearly seen. Two satellites are present on the short arms of a pair subterminally constricted, and the other two on the short arms of a submedianly constricted pair. In Fig. 50b in the lower polar group one satellite was distinctly split, indicating a behavior similar to chromosomes at this stage discussed earlier. Here it should be emphasized that the satellites are normal components of some chromosomes and form their characteristic features with permanent attachment and are not free bodies picked up by chromosomes from the surface of the nucleolus, as was suggested by Navashin (1927).

The *Paeonia* species studied had 5 pairs of chromosomes. They varied somewhat in size of chromosomes and satellites and in number of satellites. Fig. 50 represents a young seedling of *P. suffruticosa* from the Arnold Arboretum, while Fig. 51 represents a plant of the same species that has been growing at the side of the Bussey greenhouse. When slides made from these two plants were compared, a constant difference in number of nucleoli was found. The highest number reached in the greenhouse plant (Fig. 51) was always less than that of the Arboretum plant (Fig. 50). On the basis of Heitz's theory it was suspected that this discrepancy in the number of nucleoli in two different plants of the same species might be due to a difference in the number of satellites. To test this idea many cells at anaphase and metaphase were crushed by pressing on the cover glass and forcing the chromosomes to spread apart to make their satellited feature more obvious. Strikingly enough, the result was that actually the number of satellites of the plant near the greenhouse was less than in the plant of the Arboretum, three and four respectively. Fig. 51 represents an anaphase stage with three satellite chromosomes at each pole pressed flat and the chromosomes forced to spread. There was one pair of chromosomes submedianly constricted with very small satellites compared with a similar pair referred to in Fig. 50, and only one of the chromosomes with a subterminal constriction possessed a satellite corresponding in size to the ones illustrated in Fig. 50.

Figure 52 is a metaphase stage of *Paeonia Woodwardii* chromosomes. This plate also was crushed and flattened. The division at the polar constriction areas was quite distinct. There were six satellites, two more than in *P. suffruticosa* (Fig. 50), these two additional ones being on the shorter arms of a near-medianly constricted pair of chromosomes, while the other two pairs were on the same type of chromosomes as in *P. suffruticosa*. The satellites on these sub- and near-medianly constricted chromosomes were as large as the satellites of the submedianly constricted chromosomes of Fig. 50, while the ones on the subterminally constricted chromosomes were very minute and close to the end of the chromosomes and were determined only after considerable effort. Fig. 53 is from *P. Delavayi alba,* with one subterminally constricted chromosome being shown which possessed a large satellite quite comparable to the ones in Figs. 50 and 51, in contrast to the small ones in Fig. 52, varying only in the length of the achromatic thread, which was somewhat shorter. In other details the chromosomes of this species were comparable to those of *P. Woodwardii* (Fig. 52). On the basis of polar constriction the chromosomes of all *Paeonia* species reported here may be classified thus: one pair subterminal, one pair submedian, and three pairs (in varying degree) near-median.

D. ORIGIN AND BEHAVIOR OF THE NUCLEOLUS

Considerable space has been given to consideration of two important works by Van Camp (1924) and Heitz (1931a and 1931b) concerning the origin of the nucleolus in plants. Van Camp, applying Ehrlich-Biondi stain, was able to show that the nucleoli originate from the chromosomes at telophase in the form of small globules which later, by fusion, collect into one large nucleolus, while Heitz limits the origin of the nucleoli to the satellite chromosomes, specifically on and around the achromatic threads that connect either a satellite or a constricted arm with its chromosome. These two findings were subjected to an intensive study and their merits evaluated.

I. THE NUCLEOLUS IN SOMATIC CELLS. The origin of the nucleolus is best studied in longitudinal root-tip sections of plants with large chromosomes. Fig. 9 represents two sister cells from Callisia root-tip sections. Here are shown, diagrammatically, small globules of nucleolus that were differentially stained red in contrast to the pale blue color of the chromosomes which are not shown. These globules collect, undoubtedly by fusion, into two and finally one body (Figs. 10 and 11) and, as more globules are produced and added together, the nucleolus grows very rapidly and becomes constant in size during further development of the chromosomes. In general, the number of nucleoli in *Callisia* is one or two, and quite rarely three, as illustrated in Fig. 12. All these three nucleoli were homogeneously colored bright red by the Ehrlich-Biondi stain, indicating that they were of the same nature. Whenever it was possible to distinguish the outlines of the chromosomes differentially stained, it was observed that these globules were on the surface region, around and between the split chromosomes. Fig. 31 is from *Paeonia suffruticosa* (greenhouse plant). The granules were found not only where the chromosomes are more compact, but also on the arms of the chromosomes that lay outside the region of the compact mass. Here it can be clearly shown that the origin of nucleolar substance is not associated with the achromatic region of the satellite. Otherwise, assuming that these exposed arms may represent satellite arms, there should have been one granule to an arm, which is not the case. Fig. 32 is a diagrammatic representation of a similar stage, drawn on a larger scale to bring out the approximate number of these globules. Fig. 37 is from *Pinus* and represents the same very high number of small nucleolar globules. In *Polystichum* was found the same situation as in others; however, the higher number of chromosomes in this species ($2n = 60+$) making it difficult to study the satellite or secondary constriction (if present), the representation of this situation was limited only to plants in which the number of satellites and secondary constrictions was determined so as to have a sound basis for discussing satellite and nucleolar relationship. Because of the same difficulty, a species of *Arisaema* (*A. triphyllum,* collected near Pepperell, Mass.) with $2n = 60+$ chromosomes is not illustrated, but this also showed the same numerous small globular nucleoli at a stage similar to that illustrated for *Paeonia* and *Pinus* in Figs. 32 and 37 respectively.

In Fig. 29 is shown a part of a satellite chromosome from an early metaphase plate of *Paeonia suffruticosa* (Arboretum plant) to illustrate the supposedly true relationship between a satellite and the nucleolus. This small nucleolus is, perhaps, a remnant of a larger nucleolus that has not completely disappeared in the cytoplasm. It can be seen not only that this nucleolar body does not surround the achromatic thread—contrary to Heitz's theory—but also that it lies away from the thread and is found very near the satellite (there may actually be an attachment between them). Both McClintock (1931) and Burnham (1932) have shown very clearly in *Zea Mays* that the nucleolus lies attached at the side of the achromatic thread of the satellite and not around it. In *Callisia* (Fig. 22, meiotic stage) and *Paeonia* (Fig. 29) this association seems to be between the satellite and nucleolus instead of between the achromatic thread and nucleolus.

A behavior of extrusion of the nucleolus into the cytoplasm in microsporogenous cells will be more fully described below. In Pinus somatic cells not a trace of the nucleolus was found at metaphase, as was also reported by Zirkle (1931); in *Paeonia* very rarely one or two small pieces were found just before mitosis (Fig. 29); in *Callisia* often there were found one or two pieces just before (Fig. 4) and after (Figs. 16 and 17) mitosis. A similar phenomenon was observed in Polystichum root-tip sections. Fig. 45 is a diagrammatic representation of the metaphase from side view. Outside the division sphere there is a nucleolar body that is a remnant from an earlier division stage. Similarly, in Fig. 46, is shown a large nucleolus outside the nucleus which is in prophase stage. Fig. 47 shows a nucleus with a typical nucleolus from an active region. The large nucleolus in the nucleus shows a bag-like feature as if containing a number of free bodies inside a bag, while the ones outside in the cytoplasm (Figs. 45 and 46) are uniformly round and homogeneous. Besides the large nucleolus shown in Fig. 46 there are a number of small nucleolar particles, staining red like the large ones, which may aggregate into various forms, as shown in Fig. 45, and finally be extruded into the cytoplasm, where they may further coalesce into a more compact form (Figs. 45 and 46). This peculiar form of nucleoli in such small granules may be comparable to the so-called amphi-nucleoli described by Wilson (1925). No speculations are justified here as to either their analogy or homology, since the present author has not made a study of this situation in animals. However, in *Polystichum* these particles show the same behavior as is characteristic of larger nucleolar bodies, indicating that they are of the same nature as the large ones. Extrusion of nucleolar material, in part or in whole, is reported by many investigators, among whom may be mentioned

Davis (1903) and Wakayama (1930), working with fungi, Yamaha and Sinoto (1925) on some thirty species and forms of phanerogamic plants, while in some Protista (Belar, 1928) the nucleolus does not disappear at all during mitosis but divides into two which are included in the sister nuclei.

In fixed material of *Callisia,* as well as in living, in the stigma hair nucleus there often were found around the region of the nucleolus some small extranucleolar bodies (Fig. 13). When subjected to differential stain, these bodies differed in their coloring when compared with the bright red of the nucleolus,.their color being of a darker shade of red. According to Heitz (1928), these bodies are considered to be chromosome fragments, but recently this assumption was refuted by Scheuber (1932), who finds that these bodies vary in number, size, and shape and show differences in staining when compared with the nucleolus. In *Callisia* the situation was found to be identical with that reported by Scheuber. When fixed material is studied carefully, one will generally find a clear area around the nucleolus, no matter how small it may be, while around these bodies no such clear area is observable, and their staining is intermediate, though more like nucleolus than chromosomes.

Similar bodies were observed in the microspore cells of *Callisia.* Fig. 27 represents a late prophase stage with six chromosomes, a large nucleolus in the middle, and a body at the side. This body can not be confused with a satellite, since the four short chromosomes point away from it and also because of its characteristic stainability referred to before. Fig. 28 is the drawing of a metaphase with such a body at the distal end of the satellite chromosome. Again it should be stated here that this body stained characteristically and was no doubt an extranucleolar body. It seems to have a remarkable stability in size and does not disappear as the nucleolus would. Identical bodies were observed during anaphase and telophase where it could be seen that they are left outside the new nucleus in the cytoplasm, there perhaps to disintegrate eventually. Such bodies were also found in root-tip cells of *Callisia.*

II. The Nucleolus in Microsporogenous Cells. Undoubtedly the process of nucleolar origin in the telophase of archespores is the same as was described for the somatic cells of root-tip material. No favorable stage having been found, this point could not be illustrated.

Figures 14-17 are from very young microsporogenous tissue of *Callisia.* At this stage quite commonly were found large extranuclear nucleolar bodies. In Fig. 15 there are two sister cells that have developed at a parallel rate, both being at late prophase stage. At the outside of each nucleus there is a large nucleolar body in an opposite position to the corresponding one in the sister cell. Undoubtedly these two extranuclear nucleoli are remnants from a like cell shown in Fig. 14. Figs. 16 and 17 show the behavior of a nucleolus that has not been dissolved completely during the earlier stages of mitosis and is forced into the cytoplasm where it may remain for a considerable time during later stages of meiotic development, since these cells are destined to become microsporocytes. Fig. 18 represents the earliest stage of a microsporocyte after emerging from a mitotic division similar to the one shown in Figs. 16 and 17. In this, besides a large nucleolus being present among chromatic threads, one medium-sized nucleolus is found outside the nucleus in the cytoplasm probably resulting from a process illustrated in Figs. 15-17. In Fig. 23, which represents a much later stage, a nucleolus is found outside very near to the main nucleolus inside the nuclear membrane to which two chromosomes seem to be attached, while there is still another extra round body within the nucleus which is free from any such connection. Inside the cell represented in Fig. 21 there are present two such free round bodies, besides the larger nucleolus with a chromosome attached to it, that also show no connection with any chromosomes. In Yucca microspore mother cells there are either two medium-sized or one large nucleolus present inside the nucleus, as shown in Fig. 55. This figure is drawn at the nucleolus level, and not all the chromosomes are included in it. It must be emphasized here that the nucleolus is free from any chromosome attachment. The nucleolus in *Yucca* retains its size till the time of disappearance of the nuclear membrane, then it is extruded away from the division plane, sometimes one nucleolus to one side and another to the other side of the division plane, or both to one side individually, or two closely together, as shown in Fig. 56, there perhaps to disintegrate gradually. The nucleolar extrusion referred to in *Callisia* and *Yucca* may be analogous to a similar but seemingly more common behavior in *Polystichum*.

As is described above, the nucleoli that have not completely dissolved after the disappearance of the nuclear membrane are not retained in the newly-forming cells but are forced outside; so this behavior may be considered the primary rule but perhaps not the only rule. As is the case in some Protista described by Belar (1928), Christoff and Gentscheff (1932) have assumed that the nucleolus divides during mitosis, and the pieces of these nucleoli are retained in the newly-forming sister nuclei. So far most of the findings by many investigators and the present author indicate the contrary. However, in the present study there was encountered a difficulty which may be explained if it is assumed that rarely some of these pieces of nucleoli may be enclosed among the dividing chromosomes and retained in some of the nuclei.

As was pointed out, the nucleolar pieces shown outside the nucleus in Figs. 15 and 18 took a bright red color similar to the ones inside, while the free nucleoli inside the nucleus in Fig. 21, one outside and one inside the nucleus of Fig. 23, stained somewhat differently. If these free bodies are actually carried over from an earlier cell, the age of these nucleolus-like bodies may account for their reaction toward the stain, thus staining somewhat differently than the true nucleolus. They may remain there as inert particles. The above is presented as a mere suggestion, since the presence of these extra bodies is difficult to explain in the absence of experimental data. These bodies in the microsporocytes are of rare occurrence.

Another feature in *Callisia* was the presence of a small bud-like protuberance on the nucleolus, as shown in Fig. 22. A phenomenon similar to this is reported by Gates and Latter (1927), Maeda (1930), and Selim (1930). Gates and Latter find at the points of chromosome attachment to the nucleolus dark staining bodies, varying in number, size, and distribution. Maeda similarly finds small nucleolar bodies on the "mother nucleolus" and sometimes free from it in the nuclear cavity. Selim, studying meiosis in rice, finds the nucleolus budding off a "secondary nucleolus" at late diakinesis. He supports the view that the secondary nucleolus contributes material to the chromosomes, while the primary may contribute to the spindle.

Similarly, as in mitotic sister nuclei, in the sister nuclei of first meiotic division there were observed a number of small nucleolar globules (Fig. 26) which were found clearly oriented along the chromosome threads and not in the clear space of the nucleoplasm. Owing to some technical difficulties arising from poor fixation, no critical drawings of meiotic stages of *Paeonia* and *Pinus* could be presented. Only in an outline fashion the number and depth of nucleoli in *Pinus* (*P. Thunbergii*) from earliest to latest stages of meiosis are given (Figs. 39-44) to show the chromosome and nucleolus relationship. However, no radical differences from *Callisia* were observed in these plants. In *Paeonia suffruticosa* (greenhouse plant—other members of this genus were too young for the study of the meiotic phase of the problem) the nucleoli in the earliest stage numbered from six to one, while at later stages the number decreased to three to one, disappearing with the disappearance of the nuclear membrane. In *Pinus* at early leptotene (Fig. 39) and during early diplotene (Fig. 40), as many as nine nucleoli were found, the number decreasing as the development of the nucleus advances, but the volume of total nucleoli not showing any decrease. Fig. 41 is from late diplotene, while Fig. 42 is from late diakinesis stage when the nuclear membrane is disappearing or newly disappeared prior to the chromosome arrangement in a metaphase plate. Fig. 43 is one of the sister cells after the first reduction division, while Fig. 44 is after homotypic division before the nuclei go into resting stage. In these two figures the number of nucleoli reached as high as nine to twelve.

III. RELATIONSHIP OF NUCLEOLUS TO SATELLITES AND SECONDARY CONSTRICTIONS. Heitz (1931a and 1931b) has made an extensive study of satellite and secondary constriction number in *Vicia* and other plants upon which he has based a definite theory concerning the origin of the nucleolus. His conclusion is that the number of nucleoli in any species in telophase sister nuclei should correspond to the satellites and secondary constrictions there present, for he assumes and gives evidence, as was mentioned above, that each nucleolus at telophase originates on and around the achromatic thread of each satellite or secondary constriction in the form of a collar. This correlation could be more readily proved if there were never found more nucleoli than satellites and secondary constrictions characteristic of a species. However, it is significant that the number of nucleoli in the greenhouse plant of *Paeonia suffruticosa* never reached the highest number found in the Arboretum plant of the same species. In the former the highest number was found to be five (Fig. 34), while in the latter seven, which have respectively three and four satellites. Both *P. Woodwardii* and *P. Delavayi alba* have six satellites; in the former the number of nucleoli reached as high as nine (Fig. 35), while in the latter quite rarely to as high as eleven (Fig. 36). Similarly, in *Callisia* quite rarely there were found three nucleoli (Fig. 12) instead of two to correspond with the number of satellites; in *Pinus* (*P. Strobus* root-tip) the number reached as high as fourteen (Fig. 38) where there should have been only ten if there are ten achromatic areas in the twelve pairs of chromosomes. There can be no doubt that a discrepancy to Heitz's expectation exists, the cause of which should be looked for elsewhere. However, the above data clearly indicate that whenever there is an increase of satellites or secondary constrictions, there is a similar increase in the number of nucleoli. The analysis of the situation is given below.

IV. NUCLEOLAR SYMMETRY IN SISTER NUCLEI. Before I had occasion to review the literature on the nucleolus, the perfect symmetry in some sister nuclei in root-tip sections, as illustrated in Fig. 33, had impressed me, and I was led to assume that this symmetry was due to the spatial relationship existing between the chromosomes in the sister nuclei. Therefore the mirror image of some nuclei in having nucleoli similar in number, size, and position was thought to be primarily due to the proportionality of the spaces between the chromosomes of the sister nuclei; hence the symmetry of nucleoli in these cells. However, further work on this phase made it necessary to alter somewhat this original assumption.

Fig. 33 is a somewhat diagrammatic representation of the symmetry between four nucleoli in two sister nuclei. The symmetry is not only in number but also in size and position of the nucleoli. This drawing is from a root-tip section of *Paeonia suffruticosa* with the four satellites (as illustrated in Fig. 50). According to Heitz, the size of a nucleolus depends on the length of the satellite achromatic thread and its position on that of the satellite. In Fig. 33 the four nucleoli show a symmetry of number, size, and position of nucleoli but not a symmetry of the type expected according to Heitz, since in this plant two satellites are proximal and two are distal to the division poles (Fig. 50), while here one nucleolus is proximal and three are distal. There is also a size difference; the polar nucleolus in each nucleus seems to be more than the total volume of the other three nucleoli. Heitz would explain this on the basis that the increase of a nucleolus is proportional to the space around it; however, there were found many cases, as illustrated in Fig. 34, where there are small nucleoli which seem to have ample space to draw material and grow in size if Heitz's assumption is correct, but nevertheless they have remained small.

De Semet (1913) has proposed a genetic relationship between certain chromosomes and the nucleoli on the theory that nucleoli originate from certain chromosomes, and owing to this relationship he assumed the existence of a symmetrical relationship between sister nuclei. Yeates (1925) and Sprumont (1928) have supported de Semet's view, while Abele (1930) has explained this symmetry by assuming that "Die Nukleolar-substanz wird bei der Karyokinese in gleichen Mengen auf beide Tochterzellen verteilt, infolgedessen sind die Nukleolen oder Nukleolensatz beider Tochterkerne gleich gross."

It was pointed out earlier that the number of nucleoli during the early stages of nuclear development corresponded quite closely to the number of satellites present for the species in question, although quite often there were found more nucleoli than were expected according to Heitz's theory. However, since there seems to be some correlation between the number of satellites and the number of nucleoli, and also since during meiosis the nucleolus is constantly attached to a satellite chromosome pair in *Callisia* (Figs. 21-25), (Sax, 1932), in *Ranunculus* (Sorokin, 1929), and in *Zea Mays* (McClintock, 1931 and Burnham, 1932), therefore the symmetry often found between two sister nuclei in the number, size, and position of nucleoli is suggested to be primarily due to a physical relationship between a satellite and a nucleolus, and independently to corresponding spaces between the same set of chromosomes in the sister nuclei, where nucleolar globules "exuded" from the adjacent chromosome surfaces fill in. The decrease in number and increase in size of the nucleoli may come about from the increase in these interchromosomal spaces during the thinning of the chromosome threads and increase in nuclear volume as a whole, allowing these spaces to fuse into fewer and larger spaces, followed closely by the fusion of smaller nucleoli into fewer and larger ones.

V. THE NUMBER AND SIZE OF NUCLEOLI IN POLYPLOID RACES. De Mol (1926 and 1928) has made some assumptions concerning the size and number of the nucleoli in the di-, tri- and tetraploid varieties of Hyacinths and has concluded that the size of "complex nucleoli" and the number of "simple nucleoli" are proportional to the number of the chromosomes. He believes that after nucleolar globules have been formed during telophase, they fuse into a "complex nucleolus" which later fragments into two, three, or four "simple nucleoli," depending upon whether the variety is diploid, triploid, or tetraploid. The present author has observed the process of fusion between two nucleoli at prophase in living tissue but has never observed fragmentation. If fragmentation ever occurs, certainly it must be in very rare circumstances instead of being the rule. The abundance of higher number of nucleoli in early stages of nuclear development and lower number in later stages must mean one thing only, that this decrease of number and increase in volume of nucleoli come about through fusion of higher number and small sizes into lower number and larger sizes. Here may be reported the results of my own observations from diploid, triploid, tetraploid, and pentaploid *Petunia*.

The largest single nucleoli from longitudinal sections of root-tips of six plants (Table 1) were measured in order to establish the size relationship of nucleoli in the polyploid series. The first two of these plants were diploids (7Lx and $7Lnc_{2-8}$), the third a triploid ($14S_{2-28}$ × $7Lnc_{9}$)-1, the fourth and fifth tetraploids ($14S_{2-29}$ and $14L_{2-21}$) and the last a pentaploid (Trip.4 × $14S_{2-29}$). For the description of these plants the reader is referred to the author's paper on "Polyploidy in Petunia," 1931. On the slide of some of these plants there were either two or three root-tips in serial sections; in that case ten measurements were taken from each root, one from each serial section. It must be remembered that on one section there are hundreds of cells and as many or more nucleoli; therefore, even if only one measurement is taken from a section, it is from one of the largest among hundreds of nucleoli. During the measurements it was found that there was some constancy in the size of the largest nucleolus for all the sections of the same root; therefore, taking measurements from more sections of the same root-tip was not considered essential. Further, in order to make these measurements more proportionate and uniform, only the largest and circular appearing nucleoli were chosen to avoid confusion and error in the measurements. Below, in Table 1, are given the diametric measurements of nucleoli in microns.

It is apparent from Table 1 that no significant conclusion can be arrived at as to the increase in volume of nucleoli from the increase of chromosomes of diploid to polyploid series, perhaps for the following reasons: (1) the number of measurements appears to be small and (2) the races tabulated here are of mixed nature. At present, unfortunately, there are no available plants to make this study more extensive; therefore, of necessity, the above measurements had to be limited to the numbers presented in this table. Even though these numbers are perhaps small for an exact conclusion, it seems evident that in order to have a basis of comparison on this phase of the problem, one is required to choose plants of polyploid series that are of the same origin. Table 1 shows that even though the second diploid plant (7Lnc) is from a mutated bud from the first diploid (7L), the difference in their measurements is quite striking. The 4n plants and the 5n are derivatives from an entirely different race of diploid, while the 3n strain is from a cross of this 4n and the second 2n race. Perhaps in the case of 3n the small measurement in general may be explained by assuming that the second 2n plant had a diminution factor affecting at least the size of the nucleolus. The two tetraploids and the pentaploid measured practically the same and were somewhat smaller than the first diploid.

In the writer's paper on *Petunia* is discussed the origin of these polyploid strains. From an ordinary diploid race the writer obtained a tetraploid plant, and from these two strains were obtained some triploid strains, and finally a pentaploid strain was obtained by crossing a triploid with a tetraploid. From the old slides of these strains, cross sections, similar measurements of nucleoli were taken. The origin of all polyploid strains, as can be seen, is from a pure-breeding diploid plant; therefore, the measurements from these plants are expected to give more of the true picture concerning the differences in nucleolar volume (Table 2). In this case measurements were taken from ten serial sections of one root-tip from each plant.

Table 2 seems to indicate an increase in diameter of a half a micron from 2n to 3n, one micron from 3n to 4n, and a decrease of one micron from 4n to 5n. There seems to be no difference between the 3n and 5n strains. From these measurements, as from those in Table 1, it may be

Table 1

Petunia series			Nucleoli measurements in microns of ten sections from each root-tip																Range	Median
Strain	Pedigree	Root-tips	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0		
2n	7L⊗	1st								2	8								5.4–7.0	6.2
		2d														3	2	5		
		3d											4	1	1	2	1	1		
2n	$7Lnc_{2-8}$	1st			1	1	1	4	2	1									4.0–5.4	4.7
		2d				5	2	1		2										
		3d	1	6	3															
3n	$(14S_{2-28}$ x $7Lnc9)-1$	1st		2	2	4			1		1								4.2–5.8	5.0
		2d			1	1	1	1			4	2								
		3d			1	3	1	1		4										
4n	$14S_{2-29}$	1st									2	7	1						5.4–6	5.7
		2d								1	1	6	2							
4n	$14L_{2-21}$	1st										2	3	3	2				5.2–6.4	5.8
		2d							1		1	8								
5n	Trip.4 x$Tet._{2-29}$	1st											2	6	1		1		5.4–6.8	6.1
		2d								4	5	1								
		3d									2	4	2	2						

difficult to conclude whether or not there is any increase or decrease in the volume of the nucleolus along with the increase of chromosome number from diploid to polyploid series, according to de Mol's theory. However, there may be a tendency toward slight increase in nucleolar volume, due to an increase in number of chromosomes, and also some other factors may be involved in influencing the volume, as in the case of the difference between the two diploid strains in Table 1 and the equality of volume in the triploid and pentaploid in Table 2.

Table II

Petunia strain	Nucleoli measurements in microns of ten sections of one root-tip from each strain														Range	Median
	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8		
2n	1	2	4	3											5.2–5.8	5.5
3n				2	3	2	3								5.8–6.4	6.1
4n						1	3		1	1			2	2	6.2–7.8	7.0
5n				4	4	2									5.8–6.2	6.0

E. THE BEHAVIOR OF THE NUCLEOLUS IN LIVING TISSUE

An intensive observation was made on Callisia stigma hairs, kept alive either in tap water or 5% sucrose solution, in order to have a basis for determining how the fixed material differed from, or how closely it resembled, the living with regard to the behavior of the nucleolus and its relationship with the chromosomes. Entire pistils or styles with stigma hairs were placed in a drop of water on a slide and covered with a cover glass, and the material was studied under low or high power magnification with oil-immersion objective. These hairs, a few scores in number to a stigma, are fine and non-septate cells reaching a length of three millimeters, and were found to be very favorable material.

I. The Area around the Nucleolus. The nuclei and nucleoli in the hairs are considerably larger than those in some other parts of the somatic tissue, such as the style cells, where they may be one-fifth as large. The nucleoli in these cells may measure 4 to 10 microns in diameter, small in the very young and large in the hairs just before the bud opens. With a slow change of form of the nucleus, the nucleolus also changes its position and shape, perhaps owing to pressure exerted by the chromosome threads of the ever moving nucleus in the streaming cytoplasm. There was never found a clear area (separation area) between the nuclear reticulum and the nucleolus. This area appeared whenever these hairs were subjected to a disturbance by introducing a toxic fluid under the cover glass, resembling the area so commonly observed around the nucleolus, inside the nucleus, of fixed material. Based on this observation the conclusion may be drawn that the clear area around the nucleolus in the fixed material is due to fixation and therefore is an artifact. Fig. 13 represents, in an outline, a nucleus in which is shown the clear area around a vacuolated nucleolus. As a result of any slight injury, physical or chemical, the nucleolus, as if sensitized, immediately pulls itself to one side, somewhat shrunken, close to a globular extranucleolar body (if any is present). Simultaneously, similar shrinkage takes place in the nucleus, enlarging this clear area. The creation of this area seems to be primarily due to sensitizing of the protoplasm (used in a broad term) and secondly, if at all, to plasmolysis. This assumption is substantiated by the fact that, in some cases, when the hairs were treated with Lewitzky's fixative, a clear area was created immediately but very soon disappeared, and no appreciable area was left between the nucleolus and nuclear mass, while when a stronger reagent was used, such as Bouin's or Flemming's, the effect was permanent.

II. EXTRANUCLEOLAR BODIES. In the earlier part of this paper reference was made to the nature of the extranucleolar bodies in somatic cells in connection with their difference in stainability and their possible homology with extranucleolar bodies in meiotic cells. In the stigma hairs of *Callisia,* as illustrated in Fig. 13, these bodies are generally found close to the nucleolus. Whenever the hairs were subjected to toxic fluids, the nucleolus seemed always to pull itself away from the nuclear mass and to remain attached to one of these bodies like a balloon attached to a mast. No adequate explanation can be given at present as to the origin of these bodies, small in size and varying in number from one to five or more. Because of their being near the nucleolar region, they may be considered some sort of extruded material from the nucleolus, or perhaps pieces of nucleolar material that were carried and held among dividing chromosomes and were included inside the nucleus and retained there along with the nucleolus as inert pieces of nucleoli. The present author is inclined to consider the last assumption more likely than the first. In general there was only one body of this kind present in the nucleus, and often there were nuclei without it. It was said that they were always near the nucleolus, but never were any of these found fusing with the nucleolus, which may be considered indicative of their difference, although the nature of this difference is not known.

III. CHROMOSOME AND NUCLEOLAR RELATIONSHIP. One of the principal reasons for studying living tissues was to determine what relationship exists between the chromosomes and nucleolus. In the style where cells could be found in late prophase stages, the association between the chromosomes and nucleolus was identical with that illustrated in Fig. 1. Obviously the chromosomes are separate units, and many at least are free from any connection with the nucleolus. In the stigma hairs the nucleus appeared very finely granular and compact but was undoubtedly made up of fine threads which never thicken, perhaps because of the lack of cell division in these hairs. The hairs themselves grow in length as the pistil matures and dry up and die after they have played their part (if they have any) during anthesis. With the growth of the hairs there is an increase both in nucleus and nucleolus and a slight decrease (at least in nucleolus) when the hairs become exposed at anthesis.[1] These hairs were treated with fixative to bring about a clear area around the nucleolus to see if there was any close connection between these fine threads and the nucleolus. The only apparent connection which could be found was that between the extranucleolar body and the nucleolus; and sometimes, in addition to some achromatic threads, there were very rarely found threads crossing this clear area, apparently being dragged by the nucleolus, owing to the separation of the nuclear mass and the nucleolus. Therefore it seems that even at early stages there is no complete direct association between all the chromosomes and the nucleolus and that a single attachment point between a chromosome and a nucleolus can not be considered of any physiological importance.

IV. THE NUCLEOLAR VACUOLES. Contrary to Fikry's belief (1930) that vacuoles in the nucleoli are artifacts, the present author found that vacuolation was one of the most important features observed in living as well as in fixed material. At times the nucleoli in all the stigma hairs were found to be vacuolated; at other times only part of them were vacuolated; and at still other times there were stigmas with hairs none of which contained vacuolated nucleoli. It is apparent that vacuoles are not permanent features (in the strict sense of the word) of the nucleoli, but vacuolation may be considered a normal phenomenon, and

[1] As a record I should like to mention that in the case of *Callisia* the size of the nucleolus is not constant all through the plant system. In microsporogenous tissue it is constant in all cells, at all stages, measuring about 4 microns; in the stigma hairs it varies from 4 to 10 microns, depending upon the stage of growth of these hairs; in the tissue of style and stamen filaments it measures about 2 microns; while in root-tips, in regions where there is active division, the size may be about 4 microns, and in the epidermal and root-cap cells about 2 microns. In all these cases there is a correlation between size of nucleolus and nucleus. The differences here recorded may be due to difference of nutrition, so that whenever the supply of food is less, there is a corresponding diminution in the size of both nucleus and nucleolus and vice versa.

vacuoles may appear and disappear normally. To check up this assumption, stigma hairs in water cultures were kept as long as they could be kept alive to see if non-vacuolated nucleoli would become vacuolated.

For this experiment young pistils or styles with stigma hairs cut off from pistils were put in a drop of water on a slide and covered with a cover glass. They were first studied under the microscope with oil-immersion objective, the desired regions spotted, the hairs and nucleolus in its surroundings sketched, and the nucleolus measured if necessary; then the slides were put away in a petri dish. At the bottom of the petri dish was placed some wet filter paper to create a moist chamber. This method enabled me to keep some of this material alive in tap water for six days. After many trials not a single unvacuolated nucleolus had become vacuolated. Some appeared to contain vacuoles in the form of small droplets, but this proved to be a sign of degeneration which was followed by the death of the cell.

The general effect of this treatment was that in the living cells the nucleoli always showed considerable decrease in volume. One nucleolus was measured soon after the material was put on the slide. It measured 9 microns in diameter; at the end of the second day it had decreased to 6 microns, to 4 microns at the end of the third day, and to 1.8 microns at the end of the sixth day; and finally the cell died. Results of this kind would lead one to suppose that the nucleolus was used up as reserve food by the "starved" cell and hence its extreme decrease in size. However, a similar decrease was noticed in the nucleus itself but not in the same proportion (no measurements of nuclei were taken).

Analogous behavior is reported by Meyer (Guillermond et al., pp. 183-184, 1933), who believes that the nucleolus is made up of "ergastique" elements of the cell and that the volume of the nucleoli is essentially variable during the physiological state of the cell. The volume of the nucleolus increases when the cell has an abundant supply of nutritive material and diminishes when the cell is in a starving condition. In the mesophyll of *Galtonia candicans* leaves Meyer finds that there is a diminution in the volume of the nucleolus when these leaves are etiolated and states: "Si, dans la feuille vivant dans des conditions normales, on exprime ce volume par l'unite, on constate qu'il s'est abaissé à 0,38 dans une feuille maintenue à l'obscurité pendant 36 jours et à 0,18 dans une feuille maintenue à l'obscurité pendant deux mois." During the change of the albumen of *Galtonia* this author finds some interesting changes in nucleolar volume which are described thus: "dans les cellules de l'albumen jeune, les nucléoles mesurent 52,3 environ; leur volume s'accroît à mesure que l'albumen se charge de matériaux nourriciers, jusqu'au volume de 101,3; puis il diminue au moment de la formation des parois cellulaires, et surtout au moment de la germination et de l'utilisation de réserves, jusqu'à devenir insignifiant."

To supply food matter for the cells to prevent them from "starving," some material was kept in 5% sucrose solution in vials and on slides. In this solution it was possible to keep these hairs alive for eighteen days. At the end of this time, owing to the growth of yeast cells and molds of various kinds, the tissue as a whole was destroyed. The striking difference between water and sugar cultures was that while there was invariably a decrease of nucleolus in the water, there was hardly any appreciable decrease in the sugar solution, except that whenever the water under the cover glass had evaporated in part, the nucleoli were found rounded up and the flow of the cytoplasm slowed down. As soon as more water was added again, the cytoplasm increased its speed of flow, and the nucleoli took on ellipsoid or other shapes due probably to the activation of the nucleus. In one case one cell was recorded dead because there could not be observed any movement in the cytoplasm, but when water was added, it soon revived and showed cytoplasmic streaming.

It was observed that the vacuolated nucleoli did not all lose the vacuole when they were kept in sucrose solution, except in a few cells, indicating that the tissue can live normally in the sucrose solution, for a while at least, while if kept in tap water, some radical change (superficially physical) seems to take place. During these experiments, however few in number, no vacuolation, *de novo,* was observed even in the sugar solution. This was perhaps due to the somewhat abnormal condition in the sugar solution (if it is assumed that vacuolation is a normal phenomenon), since this solution can not be considered an ideal medium for tissue culture.

Some measurements were taken of nucleoli, some of which were vacuolated and some not. In all cases there was a decrease in volume in water cultures irrespective of the vacuole. Exact measurements were taken of two nucleoli, one with a vacuole and the other without. For convenience, let the vacuolated be No. 1, and the nonvacuolated No. 2. The diameter of No. 1 was 6.2 microns and that of its vacuole 5 microns. The diameter of No. 2 was also 6.2 microns. Overnight, sixteen and a half hours, the vacuole of No. 1 had disappeared, and the nucleolus had decreased in size to a diameter of 5.4 microns. The diameter of No. 2 also had decreased to 5.6 microns. The measurements after 26 hours were, for No. 1, 4 microns, and for No. 2, 4.2 microns. After some hours both cells were dead. As can be seen from these measurements, the disappearance of a vacuole does not seem to have any appreciable effect on the volume of the nucleolus. Therefore, it is perhaps safe to assume that the vacuolated region has been transformed into true nucleolar substance by some physiological phenomenon, the exact nature of which it is difficult to ascertain. This region appears to be of the same concentration as the cell sap. This assumption is based on the fact that in one case a small proplastid-like body was found inside this region and showed the same rate of Brownian movement as took place outside the nucleus.

F. GENERAL DISCUSSION AND CONCLUSIONS

I. Chromosome Morphology and Chromosome-nucleolar Relationship. Conclusive evidence is presented in the text to show that the chromosomes in somatic nuclei keep a form of linearity which remains practically constant from anaphase to the end of prophase; that there exists, in general, a permanence in position and structural form during these same stages. The facts seem to indicate that at no time of development do the chromosomes pass a period during which time they are in a granular state and thrown about in a haphazard way, later to reestablish themselves in threads, joining end to end, again to break apart into separate units as chromosomes.

There is no doubt, among those familiar with karyokinetic problems, of the individuality of chromosomes in structure. The truth of this matter seems to have been substantiated by Rable as early as 1885 and by Boveri (1909) and, more recently, by Kagawa (1926), Belar (1929), Koerperich (1930), and others. Rau (1930) states that in *Cyanotis cristata* pollen-mother-cells no continuous spireme appears to be formed at any stage. It is a fact that generally, morphologically, the chromosomes of a basic set in a species differ considerably from each other; therefore the possibility of diverse chromosomes, such as described for *Callisia* and *Paeonia*, forming a continual spireme is inconceivable. The conclusion may be that, normally, the chromosomes never join end to end. This point may be further emphasized by referring to the work of Sax and Anderson (1933) and others who show that during meiosis some chromosomes may interlock, a phenomenon which could not take place if these chromosomes were parts of the same continuous spireme thread. These facts indicate that the direct flow of nucleolar material into and through all the chromosomes, as suggested by some investigators, is an impossibility.

II. Origin and Development of the Nucleolus. It is shown that the nucleolus originates in the form of small globules on the surface of the chromosome threads during the late telophase stage, that very soon there is a close grouping of chromosomes, and that a demarcation between the chromosomes as a group and the outside cell-sap and cytoplasm is established. It is difficult to show if there is a definite nuclear membrane, as such, around the chromosome group at this period, but for practical purposes it may be assumed that such a membrane exists from the beginning of the late telophase till the time of metaphase plate formation. The method of origin of the nucleolus here reported was first described, I believe, by Van Camp (1924).

The process of nucleolar development appears to be a surface phenomenon which can be explained by assuming that a chemical reaction takes place between the substance on the surface region of the chromosomes and the nuclear sap. The nucleolus, then, may be considered a by-product of the chromosome matrix and nuclear sap and, in this sense, may be identified with the matrix, as was suggested by Marshak (1931), but with this difference, that it is the matrix which produces the nucleolar substance by going into a chemical combination with some substance in the nuclear sap. The difference is indicated by their reaction to the differential stain. From this it may be deduced that there exists a striking difference between the nuclear sap inside a nuclear membrane and the cell-sap outside this membrane, because of the fact that the nucleolus will originate and develop inside a nuclear membrane and float in the nuclear sap, while outside this membrane the nucleolus disappears and apparently is dissolved in the cell-sap. It appears, therefore, that the cell-sap has a dissolving effect on the nucleolus, the rapidity of which may differ in different species; hence the lagging of nucleolar particles in some species and the lack of it in others which may be assumed to depend on the varying strength of reaction of cell-sap in different species.

The phenomenon of lagging may also be explained on the basis that there exists a difference in rate of chromosome development during the late prophase and metaphase, varying in different species. For instance, it was noticed that the chromosomes in *Callisia* (somatic stage) are well developed by the time they are forced onto the metaphase plate, already well split, and ready to divide and move toward the division poles. In *Paeonia* and, to a lesser extent in *Pinus*, this development is not so far advanced as in *Callisia* before the nuclear membrane disappears; the chromosomes of the former species stay at metaphase considerably longer, while in the latter the separation of sister chromosomes is much more rapid. It is suggested that into the phenomenon of lagging of nucleolar particles the time element may enter. Thus, in species where the chromosomes divide more rapidly at metaphase, as in *Callisia*, more nucleolar particles are observed during the metaphase and anaphase; but rarely in *Paeonia* and never in *Pinus*, where the division of chromosomes is delayed, are these particles found.

Heitz recently has developed a theory to explain the origin of the nucleolus based on the fact that in sister nuclei there often exists a symmetry in nucleoli number, position, form, and size. He believes that there is a correlation between number and position of satellites and secondary constrictions and number and position of nucleoli in somatic cells, and that the nucleolus originates in the form of a collar around the achromatic thread that holds the satellite or secondary constriction segment to the end of the main chromosome body. Brunn (1932) indicates that *Primula seclusa* does not possess satellite chromosomes but possesses nucleoli. On the other hand, Geitler (1932) presents evidence supporting Heitz's theory by finding four nucleoli in telophase stages in a tetraploid form of *Crepis capillaris* which has four satellited chromosomes. It was mentioned in the text that the present author also found some such condition in the species where the satellite situation was thoroughly studied. For example, such correlation was particularly noticed in *Callisia* with two satellites with usually one or two nucleoli; in *Paeonia suffruticosa* in two individual plants with three and four satellites respectively with nucleoli constantly fewer in the former and more in the latter. However, it was found that this correlation was not complete, since it is decisively shown by Van Camp and by the present author that there is no localization of nucleolar development comparable to Heitz's assumption and that, even at resting stages, there are frequently found nuclei that contain nucleoli far above the number expected on the basis of Heitz's theory. It may therefore be pointed out that there are no definite nucleolus-producing chromosomes; that nucleoli may be produced in the form of small globules on the surface of every chromosome, which latter collect into larger globules; that some of these larger globules may come in contact with the satellites and remain attached there; and that finally the number of nucleolar globules may decrease to one by fusion between the globules, so that generally one large nucleolus is found by the time the chromosomes have developed to the late stage of prophase. De Mol's assumption of nucleolar fragmentation is found contrary to all observations made in all species reported here, and by no known mechanism can such a process be adequately explained; on the contrary, the present author observed in living tissue a fusion between two moderately large nucleolar bodies but has never seen any indication of fragmentation.

No adequate explanation can be offered for the origin of a protuberance (bud-like structure) at the side of the large nucleolus in meiotic stages nor for the extranucleolar bodies found both in somatic and meiotic nuclei. Based on the staining reaction, it is suggested that the extranucleolar bodies may be undissolved nucleolar particles that have been carried by the dividing chromosomes into the new nuclei, there to remain as inert particles. It is difficult to see if there may be a relationship between the bud-like protuberance and extranucleolar bodies. Until the origin of both these bodies is carefully studied from living material, it will be futile to speculate as to their origin and function. Whenever there is a similarity of reaction of nucleolar bodies inside the same nuclear membrane, this may be explained by assuming that each represents a nucleolar globule which, owing perhaps to its being attached to a satellite, is not able to fuse with another such globule into a large single one. The similarity or dissimilarity between the true nucleolus and these bodies may be intelligible if a method of differential staining is applied.

III. BEHAVIOR AND FUNCTION OF THE NUCLEOLUS. The present author is inclined to put considerable stress on the behavior of vacuolation of the nucleoli and considers this phenomenon of significance, since it was pointed out above that a large vacuole may disappear completely and yet not cause any appreciable decrease in the volume of the nucleolus. Therefore it seems more accurate to assume that instead of the nucleolus being used up as reserve food, it is concerned in the general metabolic processes of the organism as a whole. This assumption may be more validated when we consider that the nucleolus is very probably universally found in all nuclei of all organisms. An exception to this rule is found in the male gamete, as cited by Wilson (1925), Sharp (1926), Tischler (1926), and Belar (1928). This exception is probably more apparent than real, however, for it seems likely that the nucleolus is not lacking but only delayed in its development. The chromosomes for a long time remain unmodified until the male gamete reaches the female gamete, and there they seem to go through a resting stage, during which time nucleoli in small globules come to appear. This conclusion is drawn from the figure presented by Wylie (1923) of *Vallisneria spiralis* and from the studies of Sax and Edmonds (1933) and O'Mara on *Lilium* (1933).

Montgomery (1898) makes the statement that the nucleolar vacuoles in *Spirogyra* are normal structures. It was found by the present author that the nature of these vacuoles does not indicate any peculiarity characteristic only of that species but a similarity in behavior comparable to nucleolar vacuoles observed in *Callisia* stigma hairs and in all other parts of the plant. The number of nucleoli varied from a few small ones, differing among themselves in size, to a single large one. It was also noticed that thinly opened fine threads of the chromosomes surround the nucleolus, giving an appearance similar to the nucleus illustrated in Fig. 18. Conard (1931) seems to be justified in opposing some investigators who claim the origin of the chromosomes from the nucleolus. To this category may belong the findings of Kater (1928) and Faulkner (1929), who claim that nucleoli give rise directly to chromosomes.

In this connection some *Spirogyra* were treated with Ehrlich-Biondi stain which was introduced under the cover glass, and there was seen a distinct demarcation between the chromosome threads and the nucleolus. The chromosomes did not take any stain, while the nucleolus took a brick red color. It was also observed that the pyrenoids surrounded by the chloroplastic mass took a stain identical with that of the nucleolus. The similarity of staining reaction of the nucleolus and the pyrenoids may signify that they are composed of the same chemical material, or it may merely be a reaction due to their having the same electrical charge. The significance of their similarity in reaction with this stain will remain an open question until their chemical make-up is understood; therefore it is futile even to suggest that nucleoli and pyrenoids may have a homologous function, one in the nucleus and the other in the cytoplasm.

As mentioned in the introduction, according to de Mol (1926), Zirkle (1928), and Fikry (1930), the nucleolus may play a part in transmitting hereditary stimuli from the chromosomes to the cytoplasm. This theory may be justified from the fact that the nucleolus is built up from small globules that may either be exudation products from the chromosomes or formed on the surface of the chromosomes from a chemical combination between some material produced from the metamorphosing chromosomes during the late telophase or between matrix substance and material taken up from the nuclear-sap. It is shown that this "compound" product, the nucleolus, seems to stay unchanged in volume during the entire development of the nucleus, disappearing in the cytoplasm only after the nuclear membrane has vanished, or perhaps when it has gradually become freely permeable to cell-sap. The fact remains that the nucleolus disappears in the cell-sap either completely or partially, and in some cases, as in *Callisia* (Figs. 15-17), *Polystichum* (Fig. 46), and *Yucca* (Fig. 56), it may be left, in its entirety, in the cytoplasm and stay there undissolved, at least for a long time; while in other cases, as was indicated above, the nucleolus may not be dissolved in the cytoplasm at all, or may be included in the daughter nuclei, as seems to be the case in some Protista.

If the assumption is correct that the nucleolus is a compound product originating, in part, from all the chromosomes and thus containing material from all the chromosomes, and hence from all the genes, and since, excepting perhaps in the case of some Protista, this substance is dissolved in the cell-sap during or after cell division, the suggestion of the above authors that the nucleolus may play a part in transmitting hereditary stimuli from the chromosomes to the cytoplasm deserves consideration. It may be assumed that the chromosomes fundamentally as self-perpetuating bodies divide up and are carried into, and become the basis of the daughter nuclei; that the chromosomes, as such, do not directly transmit gene stimuli, but that this transmission takes place, indirectly, through the matrix substance, which may be considered a by-product of the chromosomes. If this is correct, an explanation may be offered that would perhaps help in interpreting some data presented by Riley (1932) concerning the phenomenon of self-sterility in *Capsella.* The data of this author seem to indicate that a self-sterility factor, instead of exerting its inhibitory influence over only half the number of the pollen grains, as is the case in *Nicotiana* (East, 1929), affects all the pollen grains. In the case of *Nicotiana* the factors of self-sterility may not be so inhibitory as in *Capsella;* hence the difference in these two genera. Therefore a hereditary factor of self-sterility may be transmitted through the nucleolus into the cytoplasm of the microsporocyte and through the four pollen grains resulting from the division of the microsporocyte.

The presence of a bud-like protuberance on the nucleolus in rice meiotic cells has led Selim (1930) to assume that a single nucleolus buds off a secondary nucleolus which disappears during the development of the chromosomes and the primary one at late diakinesis. He supports the view that the secondary nucleolus contributes material to the chromosomes and the primary one to the spindle formation. Budding is taken by him as a separation of the nucleolus into two different materials. Sȩthi (1930) has entertained principally the same ideas as Selim. In general, the transportation theory seems to have many followers, based on the fact that there has been found some sort of direct connection between some chromosomes and the nucleolus, and also because there has been observed a difference in stainability of chromosomes in early and late stages, a faint stain, or none, being taken in early stages and a deep stain at late stages. With this is associated the disappearance of the nucleolus by the time the chromosomes are arranged on a metaphase plate. Harper, as early as 1905, working on certain mildews, refuted the presence of direct contact between the chromosomes and nuleolus. Fikry (1930), working with *Rumex,* suggests that the connection observed between nucleolus and spireme is due to chance, and that there is no constant and fixed connection between them. The present author found no connection between the nucleolus and chromosomes in *Yucca* (Fig. 55); in *Callisia* and others the connection is only between certain chromosomes and the nucleolus.

It was asserted above that the diminution or disappearance of the nucleolus had no correlation either with the stainability of chromosomes or with building up of the chromosome mass. For this adequate explanations were offered.

The theory of the formation of the spindle by one of these nucleoli must be refuted because many investigators have observed and reported that nucleoli may be in part or completely extruded from the cytoplasm, but the chromosomes nevertheless divide, as illustrated in Fig. 56, without the assistance of the nucleolus and move toward opposite poles.

In this connection should be discussed the question of the electromagnetic quality of the nucleolus and chromosomes proposed in the following words by Zirkle (1928) as an addition to a number of theories proposed earlier by others: "The plastin, being electro-positive, changes the electro-negative spireme by flowing into it, to an electro-positive chromatin complex; thus the chromatin, which had collected at the equatorial plate as far as possible from the poles of the spindle, reverses its motion with its electrical charge and migrates to the two poles." Christoff and Gentscheff (1932) have subjected Zirkle's proposed point to a test by putting an electrical charge through a maize sprout, and find that nucleoli do show a definite charge, thus giving support to the above idea. However, it was mentioned early that in living material the present author has observed two large bodies of nucleoli fused together into one, that the nucleolus may be bodily extruded into the cytoplasm without an appreciable decrease in size, as is shown for *Yucca* in Fig. 56. Therefore, based on these two facts alone, the polarity phenomenon can not be responsible for dividing the chromosomes, because two similarly charged bodies ordinarily could not fuse into one, but would repel each other, and because in *Yucca* at least, the nucleoli being extruded into the cytoplasm, they are eliminated from playing any part in the division phenomenon.

It was stated above that the present writer is inclined to put considerable stress on the behavior of vacuolation of the nucleoli. It is very likely that the nucleolus may play a part in the organization of the cell as an organ that may primarily be concerned with assimilative processes by "imbibing" substances from the surrounding medium, converting them into material that may be utilized by the chromosomes especially for their growth and perpetuation. Schaede (1929) states that "Directe Verbindung zwischen Kernfaden und Nucleolus in dem Sinne, dass ersterer in amöboide Fortsätze des letzteren einmünde, besteht nicht, auch kein unmittelbarer Übergang von Nucleolarsubstanz in den Kernfaden," and that "In Anbetracht ihrer gegenseitigen Beziehungen ist eine Abgabe von Substanz in abgeänderter Form aus dem Nucleolus in den Kernfaden wahrscheinlich . . ." As Schaede points out, the nucleolar substance itself can not be directly involved in this process, as Van Camp and many others have believed. It was amply shown that the nucleolar body remains undiminished during the chromosome development. Its assistance in this process may be of an indirect nature during the vacuolation and devacuolation described above. The latter phenomenon may not be abrupt but simultaneous, since no appreciable decrease or increase was observed during this process.

G. SUMMARY

1. Because of the evidence offered that the chromosomes never lose their individuality in structure and position inside the nuclear membrane, it is argued that a direct flow of any nucleolar substance from one end of a chromosome through the entire spireme is not possible. In the case of *Callisia* some, but not all, chromosomes may be attached to a nucleolus. Applying a delicate differential stain (Ehrlich-Biondi), there was never found an intermediate staining at any point of any chromosome that may have come in contact with the nucleolus, and at all stages the chromosomes were stained pale blue and the nucleolus bright red, indicating that no direct interchange of material had taken place between chromosomes and nucleolus.

2. No correlation was observed between a decrease of nucleolar volume and the process of "emerging" chromosomes. The volume of nucleolus in meiosis studied in some species, once the maximum is reached during late telophase-early leptotene, remains constant all through the chromosome development.

3. The satellite and secondary constriction phenomena in *Callisia, Paeonia,* and *Pinus* are analyzed to determine the nature of satellite-nucleolar relationship. The nucleolus is found to originate on the surface of the chromosomes during telophase in the form of small globules, as Van Camp has shown, and not on a specific region (achromatic threads of satellite and secondary constriction) of satellite chromosomes, as Heitz believes; hence there were found numerous globules instead of a limited number corresponding to the number of satellites or segmented chromosomes. When there was a connection between a satellite and a nucleolus, it was found that the nucleolus is attached at

the side of the satellite instead of forming a collar around the satellite achromatic thread.

4. There was found some correlation between the number of satellites and nucleoli, but not exactly as was expected according to Heitz's theory. The symmetry between two sister nuclei in the number, size, and position of nucleoli is suggested to be due primarily to a physical relationship between a satellite and a nucleolus, and to corresponding spaces between the same set of chromosomes in the sister nuclei where nucleolar globules "exuded" from the adjacent chromosome surfaces fill in.

5. A behavior of extrusion of nucleolar particles in the species studied is described. In *Callisia* there were found small extranucleolar bodies which showed a difference in stainability when compared with the nucleolus. Their origin could not be determined. It is suggested that they may be inert nucleolar particles representing pieces of nucleoli that have been retained in the sister nuclei as lagging particles. On the side of the meiotic nucleolus there was often observed a protuberance, a bud-like growth. Its nature and relationship, if any, with some of the free nucleolar bodies observed in meiotic nuclei could not be determined.

6. A critical analysis is presented of de Mol's finding that the size and number of nucleoli vary proportionately as the number of chromosomes is increased in polyploid series. No significant correlation was found in a similar relationship studied in *Petunia.*

7. The results of some observations made from living tissue (stigma hairs of *Callisia*) are as follows: (a) A clear area around a nucleolus is an artifact which can be produced by applying toxic fluids to stigma hairs in culture. (b) Small extranucleolar bodies were often observed in the nuclei of the hairs. The nucleolus was found showing some affinity to these bodies when a clear area was artificially created. (c) No general chromosome and nucleolar connections were observed. The connections, when present, may be limited to certain chromosomes. Often specific connection was found between the nucleolus and the extranucleolar bodies. (d) The vacuole in the nucleolus is normal and not an artifact and probably appears and disappears normally. The phenomenon suggestive of vacuolation is considered by the present author to be of physiological significance. Some data are presented concerning this behavior.

LITERATURE CITED

1. Abele, K. (1930). Untersuchungen über die Nukleolen. (Protoplasma, **10**:550–567.)
2. Belar, K. (1928). Die cytologischen Grundlagen der Vererbung. (Handbuch der Vererbungswissenschaft. Lieferung 5 (1,B), Band I.)
3. ———— (1929). Beiträge zur Kausalanalyse der Mitose. III. Untersuchungen an den Staubfäden, Haarzellen und Blattmeristemzellen von Tradescantia virginica. (Zeitschr. Zellforsch. Mikr. Anat. **10**:73–134.)
4. Boveri (1909). Die Blastomerenkerne von Ascaris megalocephala und die Theorie der Chromosomen-individualität. (Arch. Zellf. **3**:181–268.)
5. Brunn, H. G. (1932). Cytological studies in Primula. (Symb. Bot. Upsal. 1.)
6. Burnham, C. R. (1932). An interchange in maize giving low sterility and chain configurations. (Proc. Nat. Acad. Sci. **18**:434–440.)
7. Christoff, M. & G. Gentscheff (1932). Untersuchungen über das Verhalten des Nukleolus während der Kernteilung. (Zeitschr. Zellforsch. Mikr. Anat. **15**:540–546.)
8. Conard, A. (1931). Il n'y a pas d' "amphinucléole" chez les Spirogyra. (Compt. Rend. Soc. Biol. **107**:923.)
9. Darlington, C. D. (1926). Chromosome studies in the Scilleae. (Jour. Gen. **16**:237–251.)
10. ———— (1932). Recent advances in cytology. (P. Blakiston's Son & Co., Inc. Philadelphia.)
11. Davis, B. M. (1903). Oögenesis in Saprolegnia. (Bot. Gaz. **35**:233–249.)
12. Dermen, Haig (1931a). Polyploidy in Petunia. (Amer. Jour. Bot. **18**:250–261.)
13. ———— (1931b). A study of chromosome number in two genera of Berberidaceae: Mahonia and Berberis. (Jour. Arnold Arb. **12**:281–287.)
14. East, E. M. (1929). Self-sterility. (Bibl. Genet. **5**:331–370.)
15. Faulkner, G. H. (1929). The early prophases of the first oöcyte division as seen in life, in Obelia geniculata. (Quart. Jour. Microsc. Sci. **73**(2):225–242.)
16. Fikry, M. A. (1930). Phenomena of heterotypic division in the pollen-mother-cells of a tetraploid form of Rumex sculatus var. typicus. (Jour. Roy. Microsc. Soc. **50**:387–419.)
17. Gates, R. R. & J. Latter (1927). Observations on the pollen development of two species of Lathraea. (Jour. Roy. Microsc. Soc. **47**:209–225.)
18. Geitler, L. (1932). Das Verhalten der Nukleolen in einer tetraploiden Wurzel von Crepis capillaris. (Planta, **17**:801–804.)
19. Guillermond, A., G. Mangenot & L. Plantefol (1933). Traité de cytologie végétale (pp. 183–184). (Librairie E. le François, Paris.)
20. Harper, R. A. (1905). Sexual reproduction and the organization of the nucleus in certain Mildews. (Publ. Carnegie Inst. Washington, No. **37**:1–92.)
21. Heitz, E. (1928). Das Heterochromatin der Moose. I. (Jahrb. Wiss. Bot. **69**:762–818.)

22. ———— (1931a). Die Ursache der gesetzmässigen Zahl, Lage, Form und Grösse pflanzlicher Nukleolen. (Planta, **12**:775–844.)

23. ———— (1931b). Nukleolen und Chromosomen in der Gattung Vicia. (Planta, **15**:495–505.)

24. KAGAWA, F. (1926). Cytological studies on Triticum and Aegilops. I. (Cellule, **37**:229–324.)

25. KATER, J. McA. (1928). Structure of the nucleolus in the root-tip cells of Nicotiana longiflora. (Univ. Calif. Publ. Bot. **14**(12): 319–322.)

26. KAUFMANN, B. P. (1931). Chromonemata in somatic and meiotic mitosis. (Amer. Nat. **65**:280–283.)

27. KOERPERICH, JOSÉPHINE (1930). Etude comparative du noyau, des chromosomes et de leurs relations avec le cytoplasme (Nothoscordum, Eucomis, Beschorneria). (Cellule, **39**:307–398.)

28. LEE, A. B. (1928). Microtomist's Vade-Mecum (p. 177). (P. Blakiston's Son & Co., Inc. Philadelphia.)

29. LEWITZKY, G. A. (1931). Review of investigations performed at the Cytological Laboratory of the Institute of Plant Industry in U. S. S. R. (Bull. Appl. Bot. Gen. in Plant-breed. **27**(1):9–17.)

30. MAEDA, T. (1930). The meiotic divisions in pollen-mother-cells of the Sweet-pea (Lathyrus odoratus L.) with special reference to the cytological basis of crossing-over. (Mem. Coll. Sci. Kyoto Imp. Univ. **5**:89–123.)

31. MARSHAK, A. G. (1931). The morphology of the chromosomes of Pisum sativum. (Cytologia, **2**:318–339.)

32. MARTENS, P. (1928). Recherches expérimentales sur la cinèse. (Cellule, **38**:67–174.)

33. MCCLINTOCK, B. (1931). Cytological observations of deficiencies involving known genes, translocations and an inversion in Zea Mays. (Mo. Agr. Exp. Sta. Res. Bull. **163**:1–30.)

34. MCCLUNG, C. E. (1928). Differential chromosomes of Mecostethus gracilis. (Zeitschr. Zellforsch. Mikr. Anat. **7**:756–778.)

35. MOL, W. E. DE (1926). The nucleolar globules regarded as bearers of stimulating or finishing materials of the genes. (Genetica, **8**:537–542.)

36. ———— (1927). On chromosomal constrictions, satellites and nucleoli in Hyacinthus orientalis. (Beitr. Biol. Pflanzen, **15**:93–116.)

37. ———— (1928). Nucleolar number and size in diploid, triploid and aneuploid Hyacinths. (Cellule, **38**:7–64.)

38. MONTGOMERY, T. H. (1898). Comparative cytological studies, with special regard to the morphology of the nucleolus. (Jour. Morphol. **15**:266–582.)

39. NAVASHIN, S. G. (1927). Zellkerndimorphismus bei Galtonia candicans Des. und einigen verwandten Monokotylen. (Ber. Deutsch. Bot. Ges. **45**:415–428.)

40. O'MARA, J. (1933). Division of the generative nucleus in the pollen tube of Lilium. (Bot. Gaz. **94**:567–578.)

41. RABL, C. (1885). Ueber Zellteilung. (Morphol. Jahrb. **10**:214–330.)

42. RAU, N. S. (1930). On reduction division in the pollen-mother-cells of Cyanotis cristata. (Jour. Ind. Bot. Soc. **9**:79–113.)

43. RILEY, H. P. (1932). Self-sterility in shepherd's purse. (Genetics, **17**:231–295.)

44. ROBERTSON, W. R. B. (1931). Chromosome studies. II. Synapsis in the Tettigidae with special reference to the presynapsis split. (Jour. Morphol. and Physiol. **51**:119–146.)

45. SAX, KARL (1932). The cytological mechanism of crossing over. (Jour. Arnold Arb. **13**:180–212.)

46. SAX, KARL, & EDGAR ANDERSON (1933). Segmental interchange in chromosomes of Tradescantia. (Genetics, **18**:53–67.)

47. SAX, KARL, & H. W. EDMONDS (1933). The development of the male gametophyte in Tradescantia. (Bot. Gaz.; in press.)

48. SCHAEDE, R. (1929). Uber das Verhalten des Nucleolus während der Kernteilung. (Protoplasma, **5**:41–54.)

49. SCHEUBER, L. M. (1932). A cytological study of Timmia cucullata. (Cellule, **41**:145–162.)

50. SELIM, A. G. (1930). A cytological study of Oryza sativa L. (Cytologia, **2**:1–26.)

51. SEMET, E. DE (1913). Chromosomes, prochromosomes et nucléoles dans quelques Dicotylées. (Cellule, **29**.)

52. SETHI, M. L. (1930). Microsporogenesis in Cassia didymobotrya. (Jour. Ind. Bot. Soc. **9**(2/3):126–139.)

53. SHARP, L. W. (1926). Introduction to cytology. Ed. 2 (McGraw-Hill Book Co., Inc. New York.)

54. ———— (1929). Structure of large somatic chromosomes. (Bot. Gaz. **88**:349–382.)

55. SOROKIN, H. (1929). Idiograms, nucleoli, and satellites of certain Ranunculaceae. (Amer. Jour. Bot. **16**:407–420.)

56. SPRUMONT, G. (1928). Chromosomes et satellites dans l'Ornithogalum. (Cellule, **38**:269–292.)

57. TISCHLER, G. (1926). Allgemeine Pflanzenkaryologie. Handbuch der Pflanzenanatomie 2, Lief. 2, Bd. II. (Verlag von Gebrüder Borntraeger, Berlin.)

58. VAN CAMP, G. M. (1924). Le rôle du nucléole dans la caryocinèse somatique (Clivia miniata Reg.). (Cellule, **34**:1–50.)

59. WAKAYAMA, K. (1930). Contributions to the cytology of fungi. II. Cytological studies in Morchella deliciosa Fr. (Cytologia, **2**:27–36.)

60. WILSON, E. B. (1925). The cell in development and heredity. (The Macmillan Co., New York.)

61. WYLIE, R. B. (1923). Sperms of Vallisneria spiralis. (Bot. Gaz. **75**:191–202.)

62. YAMAHA, G. & Y. SINOTŌ (1925). On the behavior of the nucleolus in the somatic mitosis of higher plants, with microchemical notes. (Bot. Mag. Tokyo, **39**:205–219.)

63. YEATES, J. S. (1925). The nucleolus of Tmesipteris tannensis, Bernh. (Roy. Soc. Proc. **98**:227–244.)

64. ZIRKLE, C. (1928). Nucleolus in root-tip mitosis in Zea Mays. (Bot. Gaz. **84**:402–418.)

65. ———— (1931). Nucleoli of the root-tip and cambium of Pinus Strobus. (Cytologia, **2**:85–105.)

EXPLANATION OF PLATES 64 TO 67

All sections are longitudinally cut.

Figs. 1–28 *Callisia repens*

Fig. 1. Method: Lewitzky fixation; stained in iron-alum-haematoxylin. Stage: prophase, oblique view, nucleolus in center, one probable attachment between nucleolus and chromosome.

Fig. 2. Method: same as in Fig. 1. Stage: prophase, side view (nucleolus not shown).

Fig. 3. Method: Lewitzky + 0.25% saponin; stained in i.a.h. first; destained and restained in Ehrlich-Biondi mixture. Stage: prophase drawn on nucleolar level, chromosomes stained homogeneously pale blue and nucleolus bright red.

Fig. 4. Method: same as in Fig. 3. Stage: metaphase with two nucleolar pieces.

Fig. 5. Method: same as in Fig. 3, before destaining. Stage: metaphase, the satellite chromosome pair, fully split; one of the sister chromosomes on the left shows a further split.

Fig. 6. Method: same as in Fig. 3. Stage: late anaphase, chromosomes homogeneously pale blue.

Fig. 7. Method: same as in Fig. 5. Stage: an anaphase chromosome with split and twist.

Fig. 8. Method: Lewitzky fixation; stained in crystal-violet-iodine. Stage: split and twisted chromosomes from late anaphase.

Fig. 9. Method: same as in Fig. 3. Stage: telophase; diagrammatic presentation of nucleolar globules.

Fig. 10. Method: same as in Fig. 3. Stage: telophase; two small nucleoli shown.

Fig. 11. Method: same as in Fig. 3. Stage: telophase, with one nucleolus larger than in Fig. 10.

Fig. 12. Method: same as in Fig. 1. Stage: interphase, with three nucleoli (the nucleoli were tested with E.-B. stain; all took bright red color).

Fig. 13. Method: same as in Fig. 1. Stage: dormant stage nucleus in stigma hair, a vacuolated nucleolus with clear area around and four extranucleolar bodies.

Fig. 14. Method: 1-3 minutes Carnoy solution, followed by Lewitzky fixative; stained in c.v.i., destained and restained in E.-B. Stage: prophase from a cell of microsporogenous tissue with two nucleoli drawn from the level of the nucleoli.

Fig. 15. Method: same as in Fig. 14. Stage: prophase of two microsporocyte sister cells, each with an extranuclear nucleolus, drawn from the level of the nucleoli.

Figs. 16 and 17. Method: same as in Fig. 14. Stage: late anaphase of a microsporogenous cell division, with median and polar lagging nucleoli, respectively.

Fig. 18. Method: Fixative same as in Fig. 14; stained in i.a.h. Stage: early leptotene, with a normal nucleolus inside the nucleus and one lagging nucleolus outside.

Fig. 19. Method: same as in Fig. 14. Stage: leptotene, with two small normal nucleoli, one with a small vacuole.

Fig. 20. Method: same as in Fig. 14. Stage: leptotene, with a large normal nucleolus.

Fig. 21. Method: 1-3 minutes Carnoy, followed by Zirkle (1928) No. 4; stained in i.a.h.; later destained and restained in E.-B. Stage: pachytene, one large normal nucleolus with chromosome attachment and two extra smaller nucleoli which stained somewhat differently. (Some of these nucleoli were in the form of a bubble with a very thin membrane stained a somewhat lighter color; hyaline inside.)

Fig. 22. Method: same as in Fig. 21. Stage: pachytene, with a bud-like protuberance at the side of the nucleolus; a satellite chromosome attached to the nucleolus.

Fig. 23. Method: same as in Fig. 21. Stage: Pachytene, with one outside and one inside additional nucleoli, which are of the same nature as those indicated in Fig. 21.

Fig. 24. Method: 1-3 minutes Carnoy, followed by Lewitzky; stained in i.a.h. Stage: pachytene; nucleolus with four small vacuoles, dark stain in the nucleolar substance, somewhat hyaline in the marginal portion.

Fig. 25. Method: Lewitzky fixation; stained in i.a.h. Stage: diakinesis; at the side of the nucleolus a satellite (?) attached. (Chromosome pair shown in outline.)

Fig. 26. Method: same as in Fig. 25. Stage: interphase of first meiotic division, showing globular nucleoli. (Chromosomes could not be shown because of their very faint color; however, these globules were very closely associated with the chromosome threads.)

Fig. 27. Method: same as in Fig. 25. Stage: microspore prophase with a large nucleolus with one probable chromosome attachment, and an extranucleolar body below a long doubled-up chromosome.

Fig. 28. Method: same as in Fig. 25. Stage: microspore metaphase, with one satellite chromosome and an extranucleolar body near the distal end of the same.

Figs. 29 and 30 *Paeonia suffruticosa* (Arboretum plant)

Fig. 29. Method: Lewitzky fixation; stained in c.v.i. Stage: metaphase; a part of a satellite chromosome to which a small piece of nucleolus is attached.

Fig. 30. Method: same as in Fig. 29. Stage: anaphase; parts of chromosomes which are split and show spiral twists.

Fig. 31 *Paeonia suffruticosa* (greenhouse plant)

Fig. 31. Method: same as in Fig. 3. Stage: telophase, where small bright red globular nucleoli were seen along pale blue chromosome threads.

Figs. 32 and 33 *Paeonia suffruticosa* (Arboretum plant)

Fig. 32. Method: same as in Fig. 29, but destained and restained in E.-B. Stage: telophase, sister nuclei. Small bright red globular nucleoli are presented in a diagrammatic way to show approximate number of these globules.

Fig. 33. Method: same as in Fig. 29. Stage: late telophase, showing a symmetry in two sister nuclei in the number, position, and size of nucleoli. Fine chromosome threads are shown diagrammatically arranged in linear fashion. Four-satellited plant with four nucleoli. The number of nucleoli may be as high as seven and possibly more.

Fig. 34 *Paeonia suffruticosa* (greenhouse plant)

Fig. 34. Method: same as in Fig. 3. Stage: interphase of sister cells, each nucleus of which possesses five nucleoli; no symmetry of size and position. Three-satellited plant with five nucleoli.

Fig. 35 *Paeonia Woodwardii*

Fig. 35. Method: Lewitzky fixation; stained in E.-B. Stage: interphase. Six-satellited plant with nine nucleoli.

Fig. 36 *Paeonia Delavayi alba*

Fig. 36. Method and stage: same as in Fig. 35. Six-satellited plant with number of nucleoli as high as eleven, generally less than this number.

Figs. 37 and 38 *Pinus Strobus*

Fig. 37. Method: Lewitzky fixation; stained in E.-B. Stage: telophase; sister nuclei with numerous small nucleolar globules.

Fig. 38. Method: same as in Fig. 37. Stage: interphase; only nucleoli shown. Ten-secondary-constricted plant with as high as fourteen nucleoli.

Figs. 39-44 *Pinus Thunbergii*

Fig. 39. Method: Lewitzky fixation; stained in E.-B. Stage: early leptotene with nine nucleoli.

Fig. 40. Method: Lewitzky fixation; stained in E.-B. Stage: early diplotene with nine nucleoli.

Fig. 41. Method: same as in Fig. 40. Stage: late diplotene with six nucleoli.

Fig. 42. Method: same as in Fig. 40. Stage: beginning of metaphase with four nucleoli.

Fig. 43. Method: fixation same as in Fig. 40, but stained in c.v.i Stage: interphase after meiotic division. Only one of the two sister nuclei shown possesses nine nucleoli.

Fig. 44. Method: same as in Fig. 43. Stage: late telophase of tetrad formation, number of nucleoli varying from eight to twelve.

Figs. 45-47 *Polystichum acrostichoides*

Fig. 45. Method: Lewitzky fixation; stained in E.-B. Stage: metaphase, with small nucleolar globules in the division region, and one outside this region, remnant of an earlier cell division.

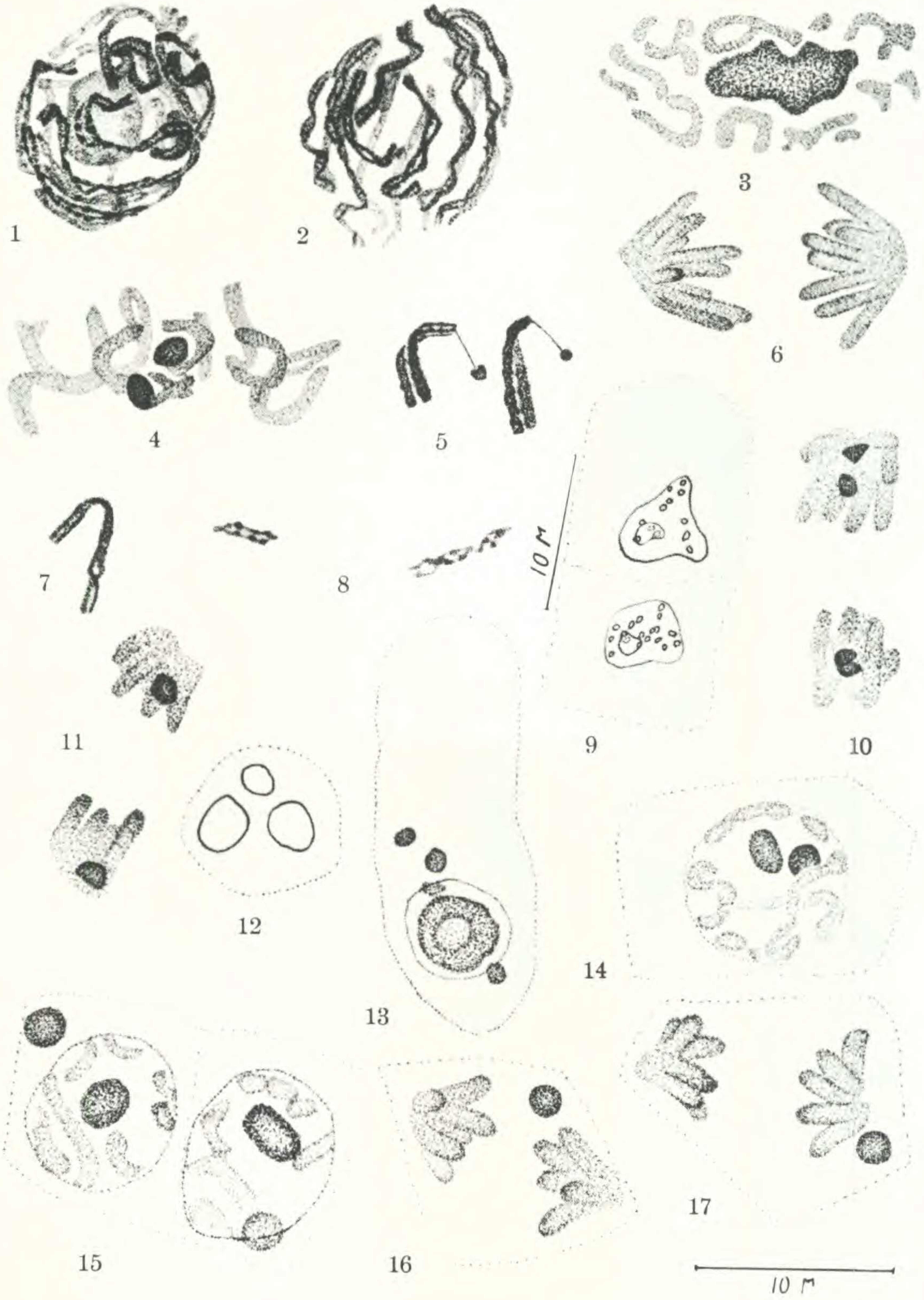
Fig. 46. Method: same as in Fig. 45. Stage: interphase from periblem marginal region with a large nucleolus inside, bag-like, containing darker staining particles; similar kinds free from the "bag." One large round nucleolar body stained darker like the small ones outside the nucleus, this body being a remnant of an earlier cell division.

Fig. 47. Method: same as in Fig. 45. Stage: a nucleus in early prophase with a single bag-like nucleolus containing darker staining smaller particles. This figure is from a more actively dividing region.

Figs. 48 and 49 *Callisia repens*

Fig. 48. Method: Lewitzky fixation + 0.25% saponin; stained in i.a.h. Stage: metaphase plate showing the structural differences of the chromosomes.

Fig. 49. Method: Navashin fixation, half strength; stained in c.v.i. Stage: anaphase.



ORIGIN AND BEHAVIOR OF THE NUCLEOLUS IN PLANTS

THE HELIOTYPE CORP. BOSTON